CORSO INTEGRATO DI GENETICA

a.a. 2010-2011

12/10/2011

Lezioni 9 e 10

I polimorfismi del DNA

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POLIMORFISMO

La presenza nella popolazione di due o piu' varianti (alleli, fenotipi, varianti di sequenza, varianti di struttura cromosomica) con frequenze significative.

Un locus e' considerato polimorfico se presenta almeno due alleli dei quali il piu' raro ha una frequenza maggiore dell' 1%, tale che la frequenza di eterozigoti per tale allele e' maggiore del 2% .

- **Mutazione** = variazione della sequenza nucleotidica rispetto ad una sequenza di riferimento
  - Effetti evolutivi = neutra, vantaggiosa, svantaggiosa
  - Patologica = determina insorgenza di una malattia
- **Polimorfismo** = mutazione con frequenza >1% nella popolazione

POLIMORFISMI NOTI

Gruppi sanguigni	~20 loci	1910-1960
Proteine del siero	~30 loci	1960-1975
Tipi tissutali HLA	1 locus (aplotipo)	1970-

RFLP del DNA	$>10^5$	1975-
VNTR del DNA o minisatelliti	~ 6000	1985-
VNTR del DNA o microsatelliti	~ 250.000	1989-
SNP del DNA	$> 10^7$	1998-

RFLP

Restriction **F**ragment **L**ength **P**olymorphism

Polimorfismo di lunghezza dei frammenti di restrizione

RSP

Restriction Site Polymorphism

Polimorfismo per il sito di restrizione

- Gli alleli differiscono per la presenza o assenza di un sito riconosciuto da una specifica endonucleasi di restrizione

- Polimorfismi biallelici
- Ereditarieta' mendeliana
- (Southern blotting)
- PCR

A

C>A

GAATTC

Sito per EcoRI

GEL ELETTROFORESI

A/A *A/B* *B/B*

Lunghezza

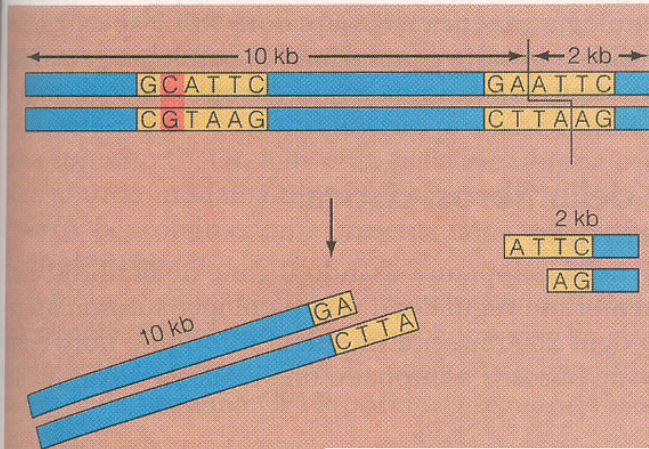
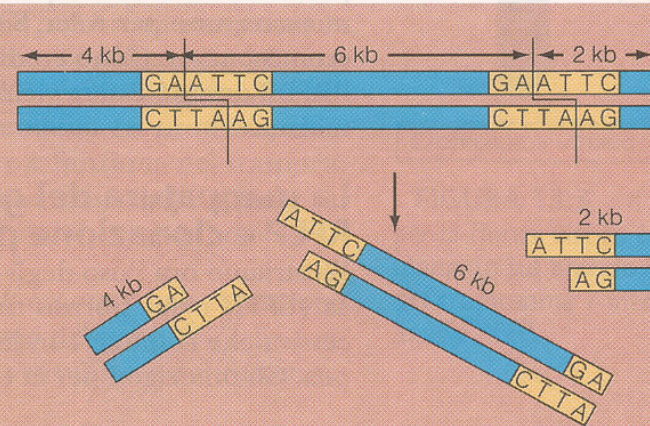
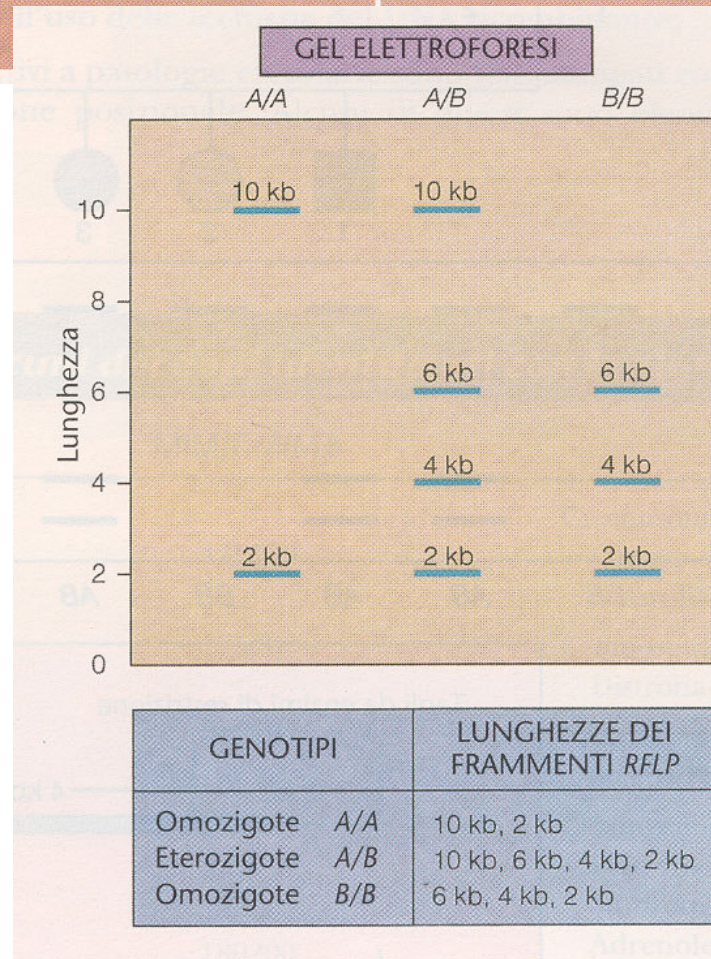
10 kb 10 kb

6 kb 6 kb

4 kb 4 kb

2 kb 2 kb 2 kb

10, 8, 6, 4, 2, 0

GENOTIPI		LUNGHEZZE DEI FRAMMENTI *RFLP*
Omozigote	*A/A*	10 kb, 2 kb
Eterozigote	*A/B*	10 kb, 6 kb, 4 kb, 2 kb
Omozigote	*B/B*	6 kb, 4 kb, 2 kb

SNP

Single Nucleotide Polymorphism

Polimorfismo di sequenza di un singolo nucleotide

- Gli alleli differiscono per la differenza di un solo nucleotide

Polimorfismi biallelici

Ereditarieta' mendeliana

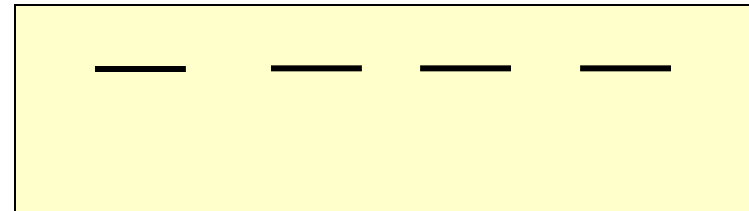
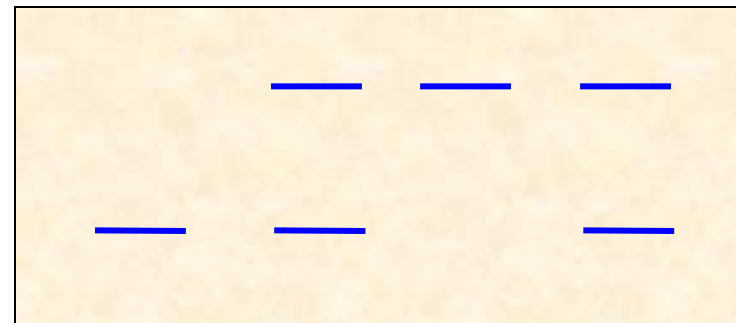
Possono essere riconosciuti da una endonucleasi di restrizione oppure no

Tipizzati in grande scala con sistemi automatizzati (microarray o chip)

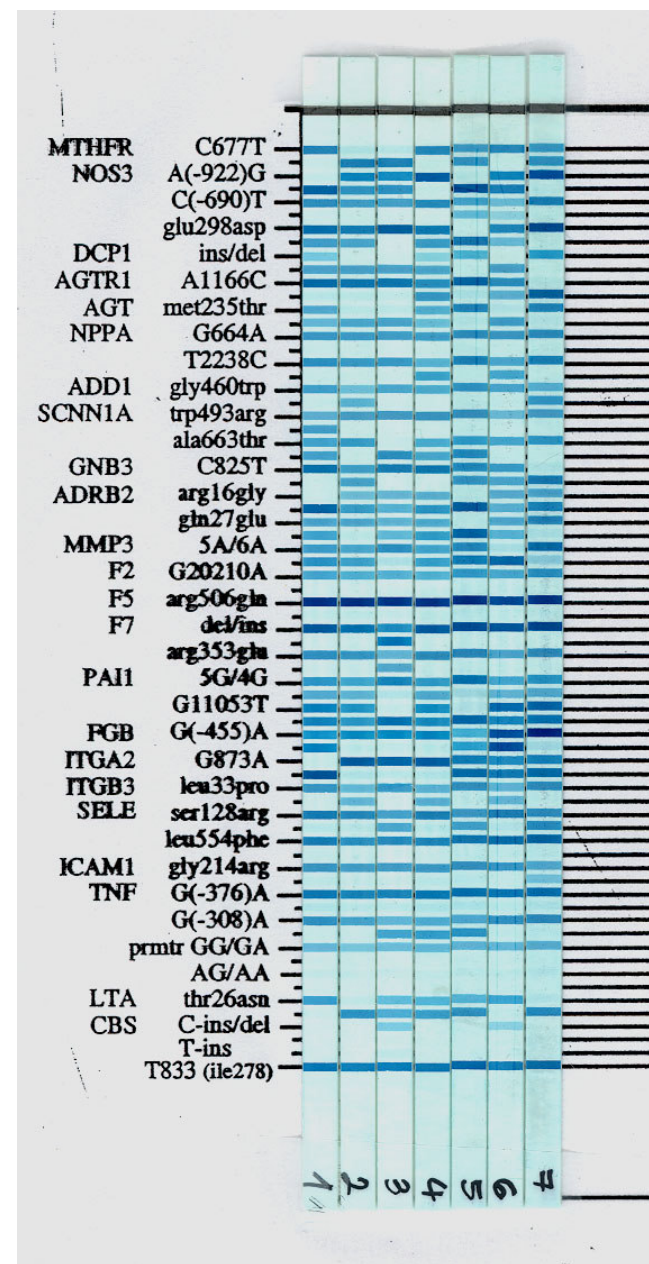
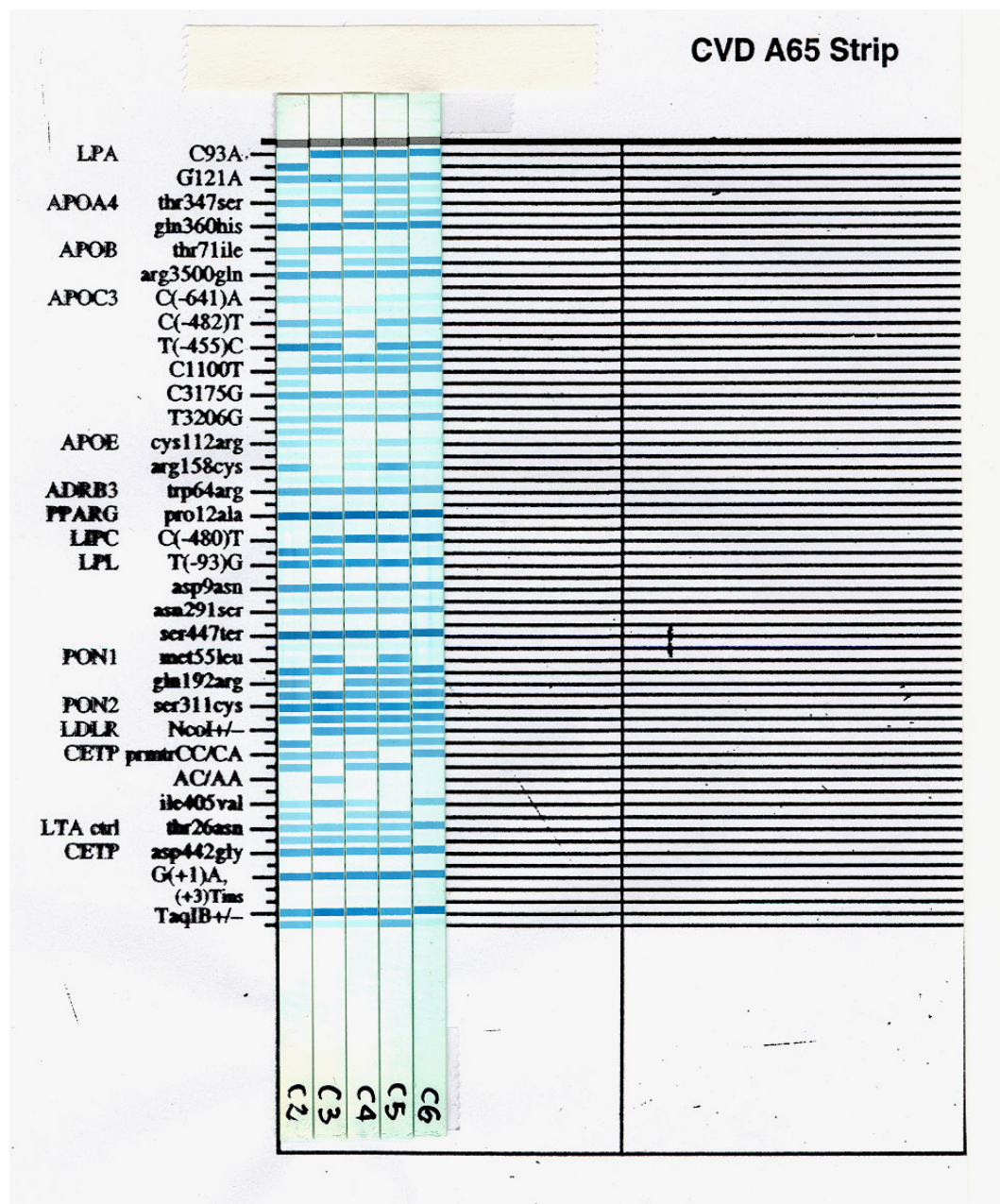
SNP non riconosciuti da enzimi di restrizione

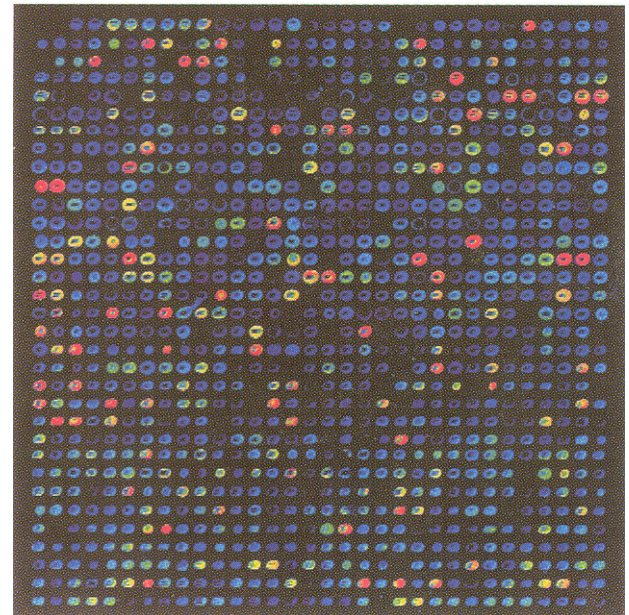
Sequenza 1 ....C...........

Sequenza 2 ....T..........

PCR + elettroforesi su 4 individui

Ibridazione con sonde oligonucleotidiche allele specifiche

Sonda 1 (C)

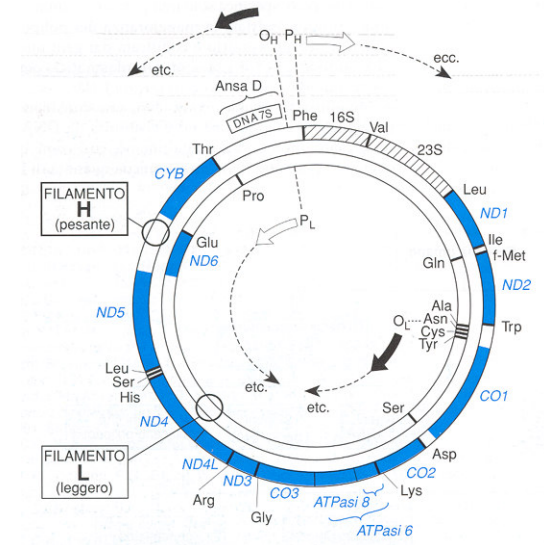
Sonda 2 (T)

2/2	1/2	1/1	1/2
T/T	C/T	C/C	C/T

Roche Molecular Systems (Alameda, CA)

Microarray

500k

Polimorfismi del DNA mitocondriale

Localizzazione

Ansa D (regione non codificante genoma mt), elevata variabilità di sequenza

Utilità

- studi antropologici ed evolutivi → risalire alle origini e parentele del genoma umano
- identificazione individuale → nonne materne di bimbi orfani (genitori scomparsi durante la dittatura militare in Argentina)

VNTR

Variable **N**umber of **T**andem **R**epeat
Numero variabile di ripetizioni in tandem

- Gli alleli differiscono per il n° variabile delle unità ripetute in tandem

- Ereditarieta' mendeliana
- Molti alleli
- Elevata eterozigosità

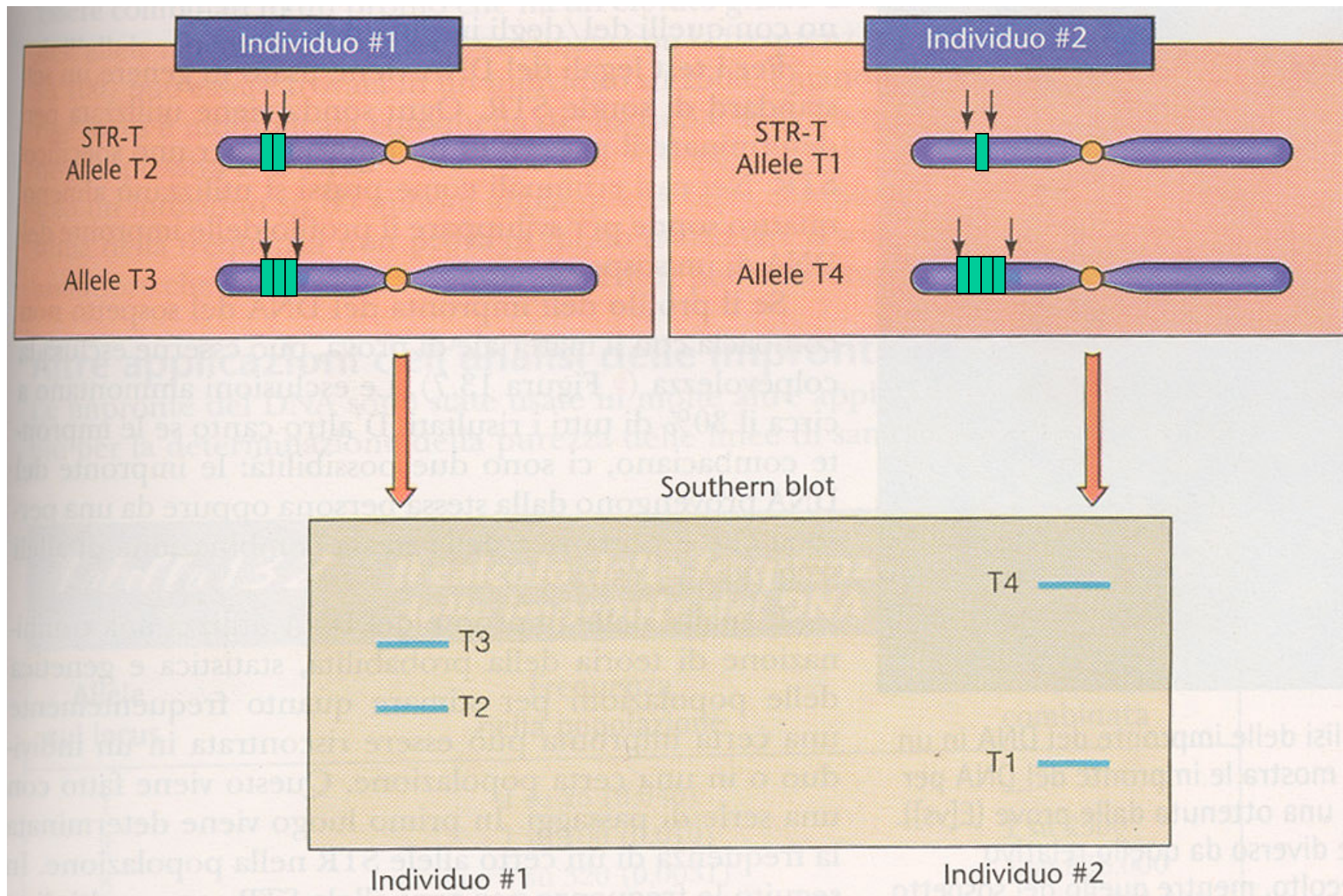
MINISATELLITI

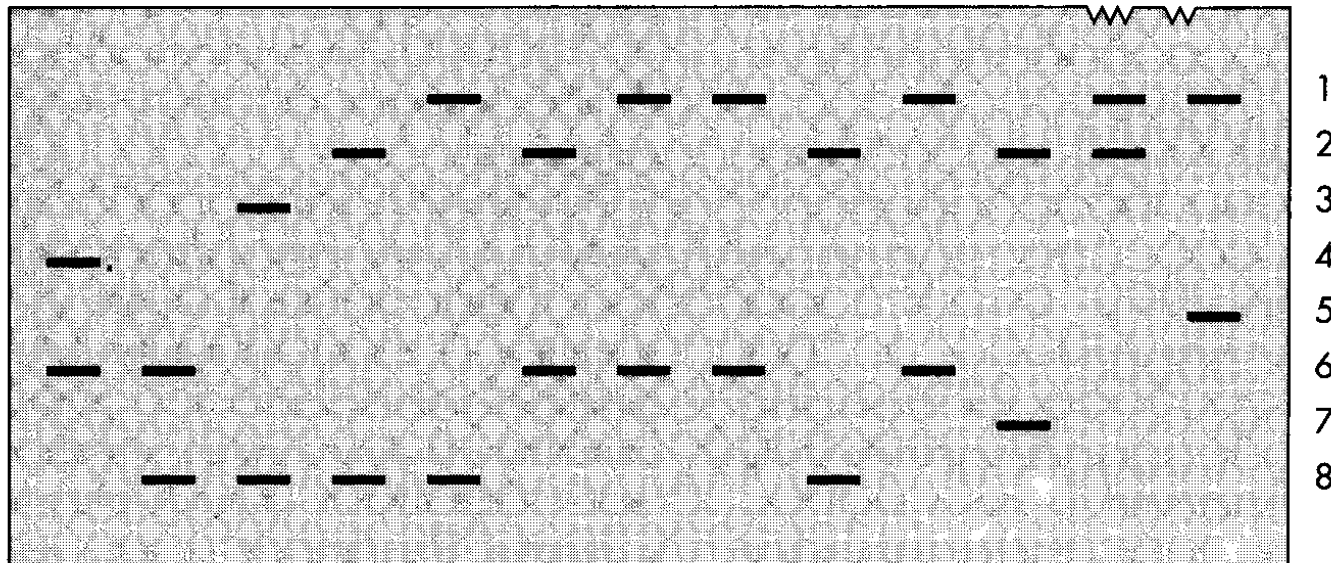
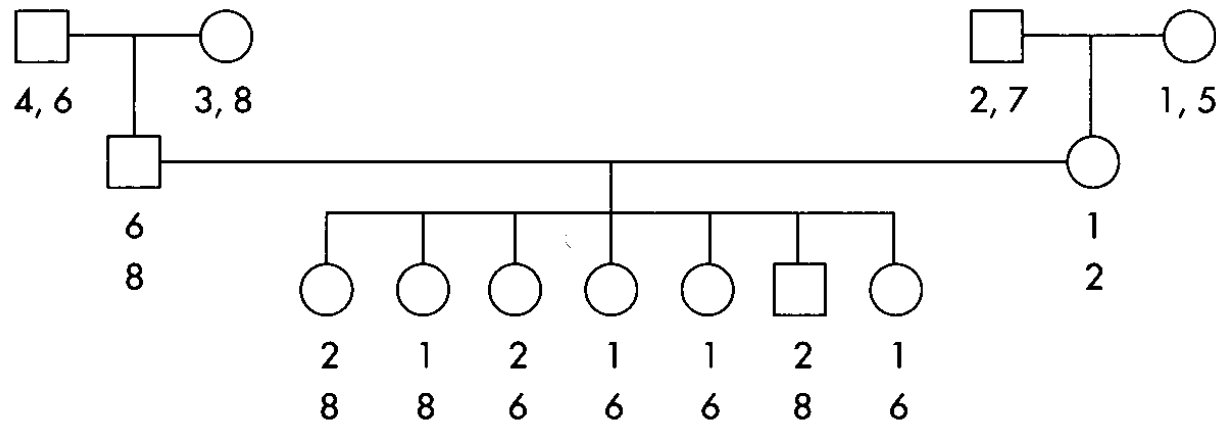
Unità ripetuta: 20bp→centinaia bp
Dimensione alleli: poche centinaia →kb
Localizzazione subtelomerica

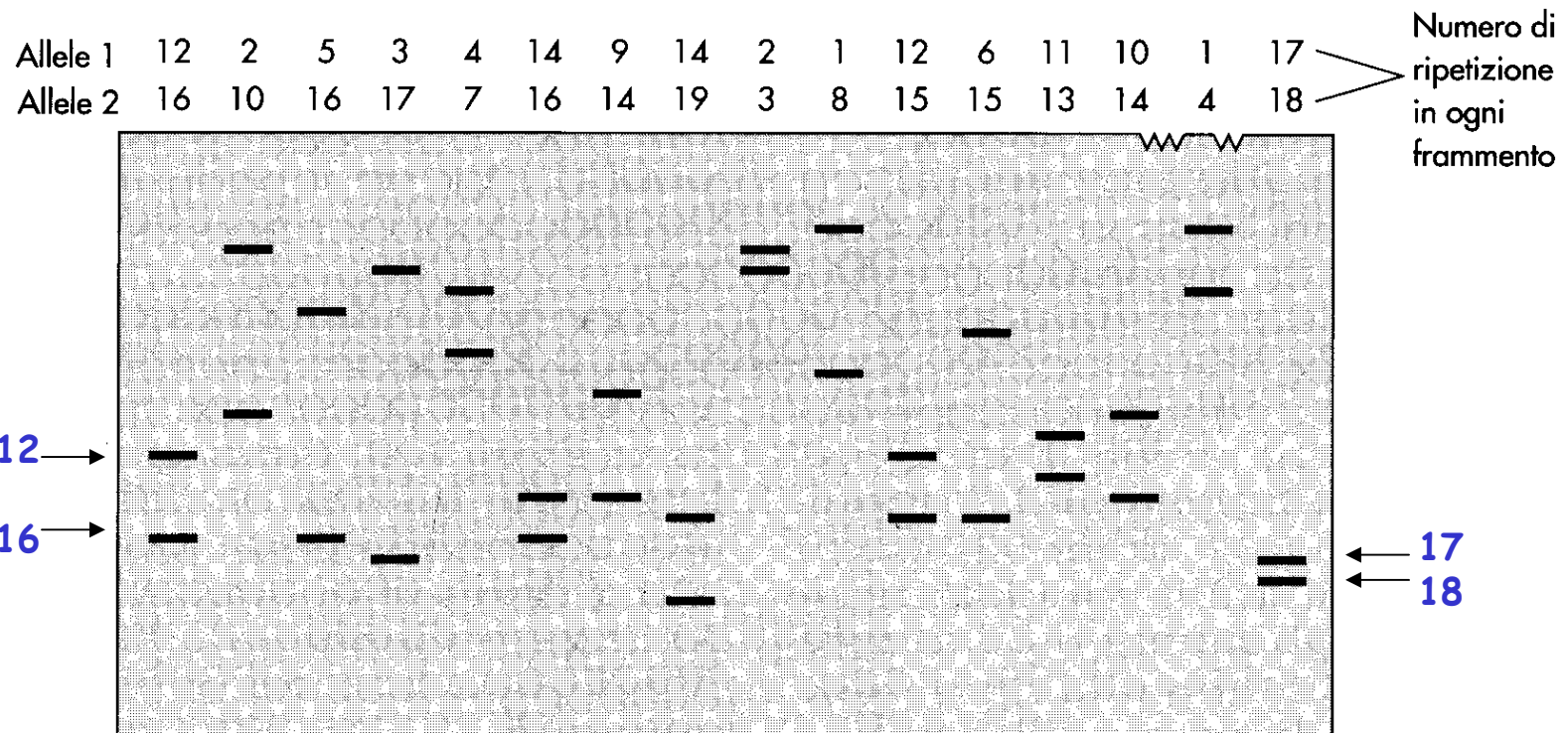
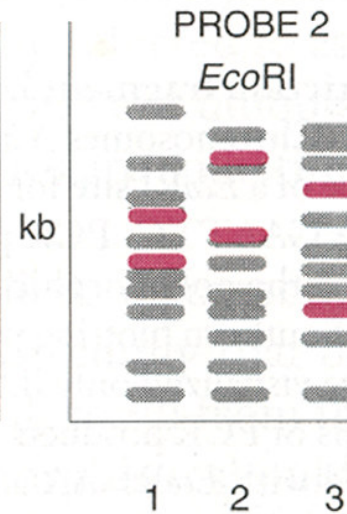
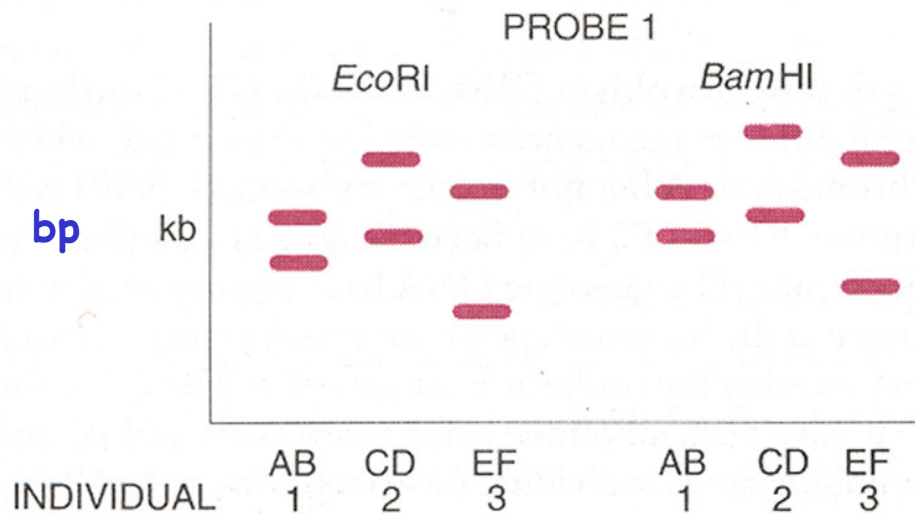
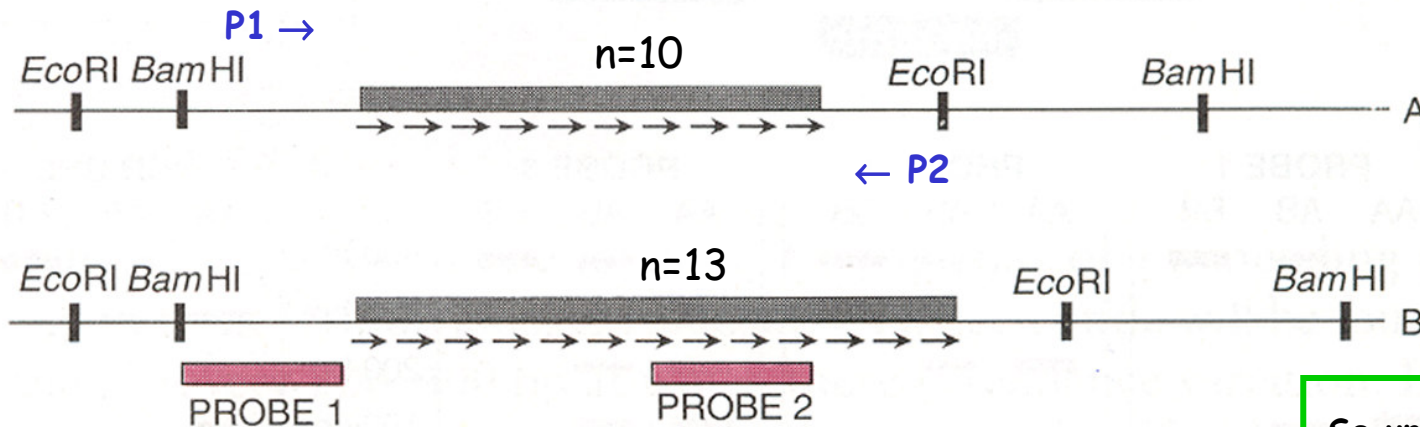
MICROSATELLITI

SSLP simple sequence length polym.
SSR single/simple sequence repeat
STR short tandem repeat

Unità ripetuta: 2-4 bp
Dimensione alleli: 70-400 bp
Ben distribuiti nel genoma
Tri- e tetra-nucleotidici
Multiplexing / automazione

Individuo #1
STR-T
Allele T2
Allele T3
Individuo #2
STR-T
Allele T1
Allele T4
Southern blot
T3
T2
T4
T1
Individuo #1
Individuo #2

Come vengono ereditate le VNTR?

Secondo le modalità delle leggi di MENDEL

16 individui

19 alleli con VNTR D2S44

Analisi di VNTR mediante S.B. e PCR

VNTR

DNA fingerprinting

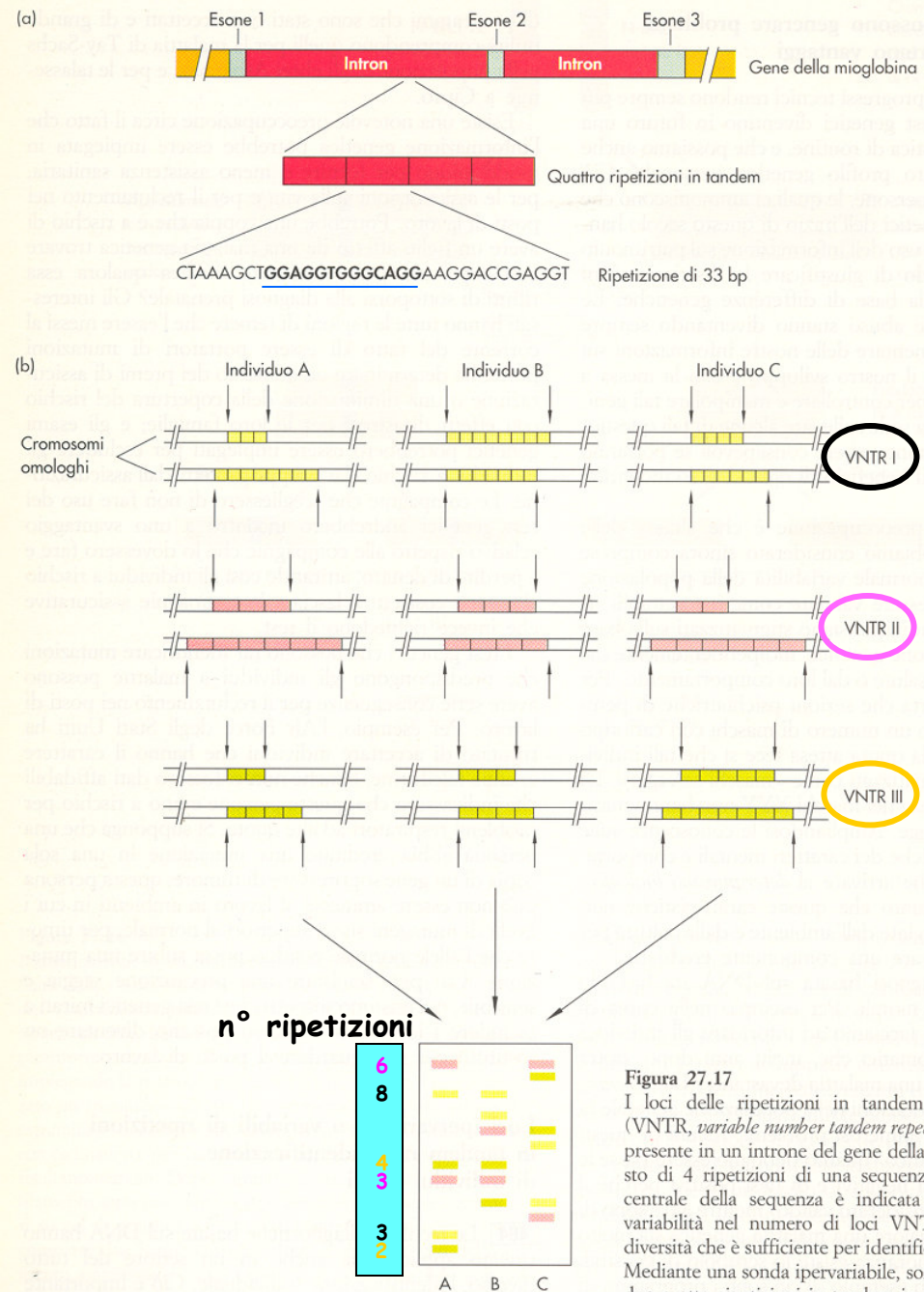



Figura 27.17
I loci delle ripetizioni in tandem in
(VNTR, *variable number tandem repeat*).
presente in un introne del gene della mio
sto di 4 ripetizioni di una sequenza di
centrale della sequenza è indicata in
variabilità nel numero di loci VNTR p
diversità che è sufficiente per identificare i
Mediante una sonda ipervariabile, sono st
due a otto ripetizioni in tandem in tre lo

Meccanismi causa dei Polimorfismi

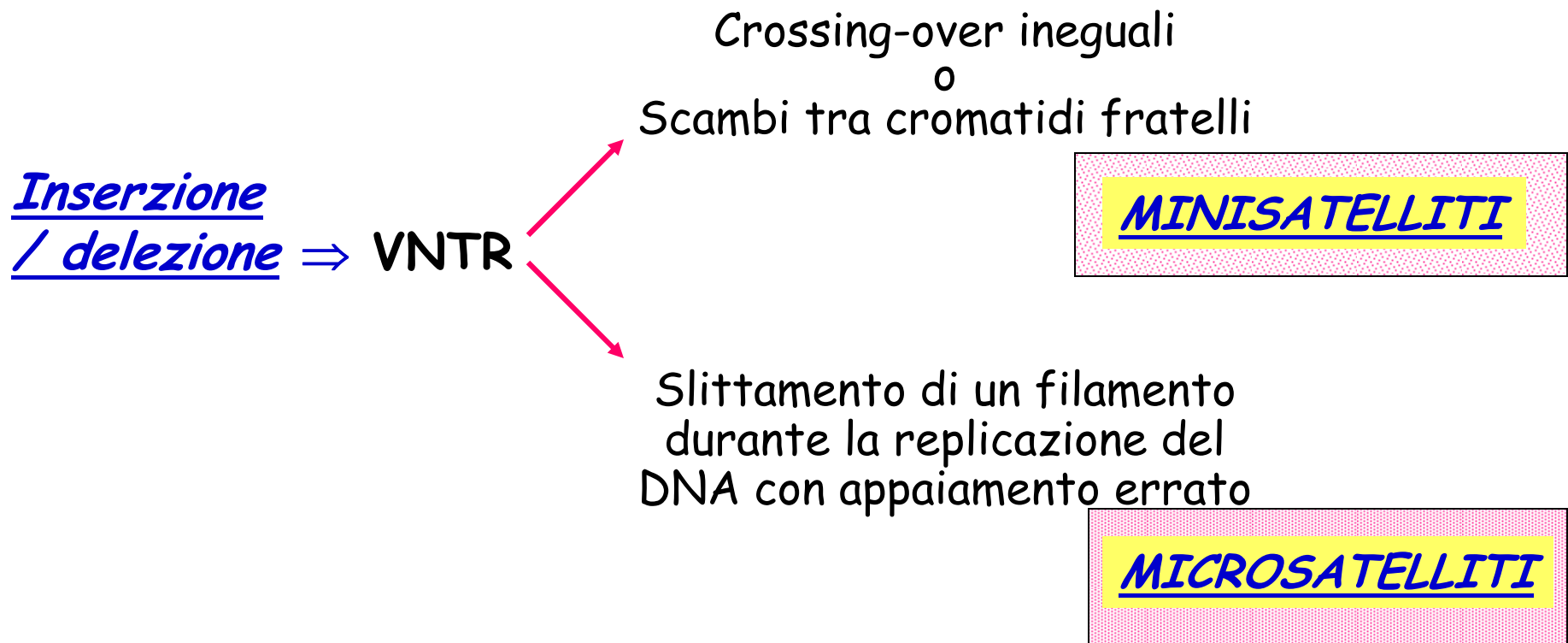
Mutazioni puntiformi ⇒ **RFLP classico, SNP**

Inserzione / delezione ⇒ **VNTR**

Crossing-over ineguali
o
Scambi tra cromatidi fratelli

MINISATELLITI

Slittamento di un filamento durante la replicazione del DNA con appaiamento errato

MICROSATELLITI

Duplicazione

RFLP	SNP	VNTR
Sito restrizione	un nucleotide	n°ripetizioni tandem

E. mendeliana	E. mendeliana	E. mendeliana
S.B. PCR	RDB - RLB seq.automatici microchip	S.B. PCR seq.automatici

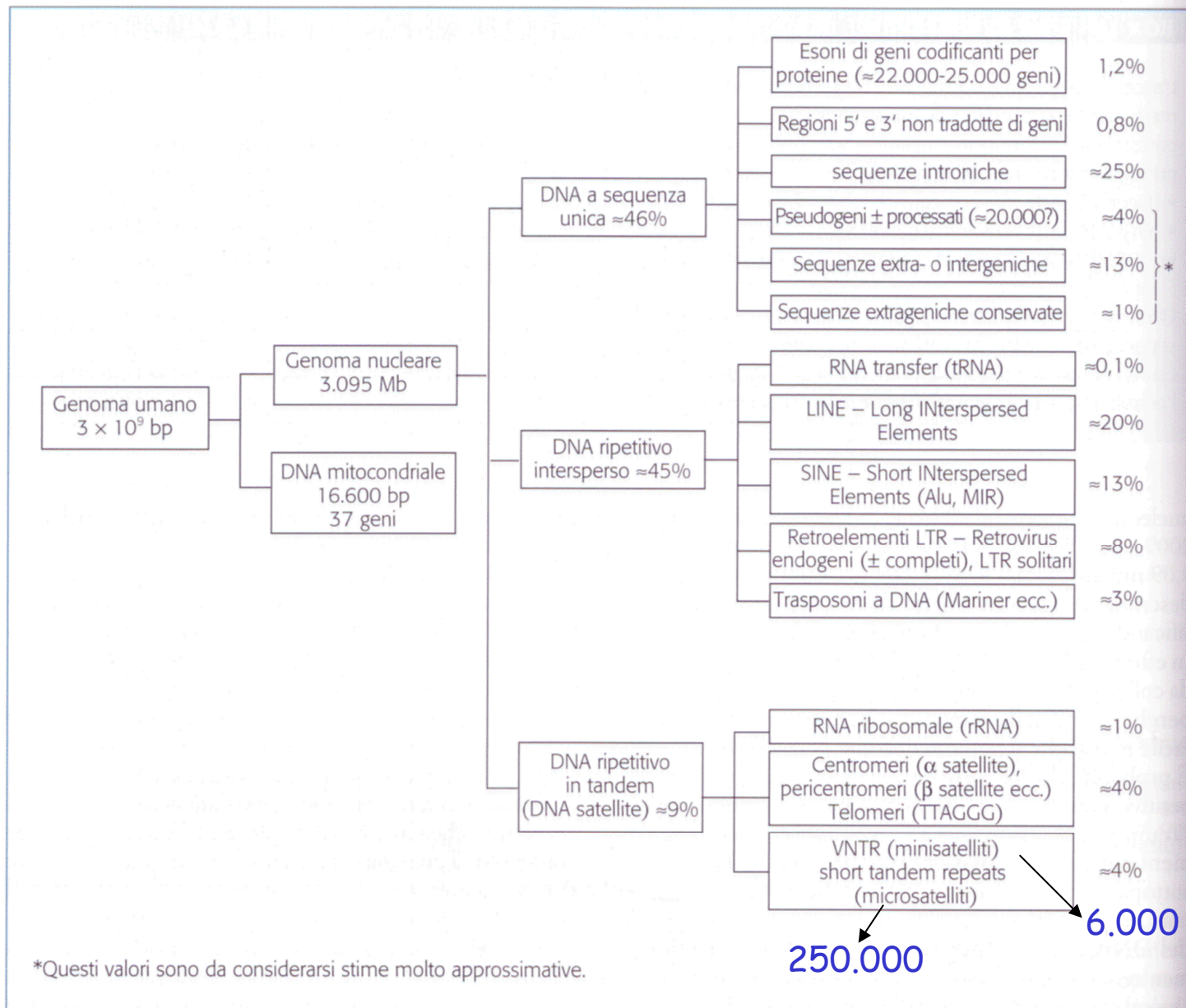



FIGURA 1.11 - Struttura del genoma umano. Il genoma nucleare è stato ripartito nelle tre componenti principali a seconda del numero di copie presenti (a sequenza unica, ripetitivo intersperso, ripetitivo in tandem).

Tabella 1.1 Il genoma umano*

Cromosoma	Lughezza (bp)	Geni codificanti proteine	Densità (geni/Mb)	Geni codificanti RNA	SNPs
1	247.199.719	2.368	9.6	457	791.897
2	242.751.149	1.511	6.2	273	758.029
3	199.446.827	1.208	6.1	240	635.618
4	191.263.063	900	4.7	186	654.646
5	180.837.866	1.007	5.6	205	568.721
6	170.896.992	1.202	7.0	197	640.584
7	158.821.424	1.104	7.0	204	546.282
8	146.274.826	813	5.6	152	487.008
9	140.273.252	1.013	7.2	158	497.975
10	135.374.737	901	6.7	147	514.966
11	134.452.384	1.467	10.9	209	503.529
12	132.289.534	1.161	8.8	205	468.093
13	114.127.980	397	3.5	104	363.494
14	106.360.585	744	7.0	202	290.746
15	100.338.915	752	7.5	245	282.234
16	88.822.254	987	11.1	113	320.728
17	78.654.742	1.321	16.8	184	256.852
18	76.117.153	320	4.2	93	267.227
19	63.806.651	1.525	23.9	124	206.202
20	62.435.964	651	10.4	106	289.087
21	46.944.323	297	6.3	32	149.076
22	49.591.432	578	11.7	64	171.587
X	154.913.754	986	6.4	213	392.586
Totale	**3.021.995.526**	**23.213**	**7.7**	**4.113**	**10.057.167**
Y	57.772.954	115	2.0	24	36.259

***** Il genoma umano è ripartito in 23 coppie di cromosomi, contenenti un numero variabile di geni. Accanto al numero di ciascun cromosoma è riportata la lunghezza in paia di basi (bp), il numero di geni codificanti proteine identificati su ciascuno di essi, la densità genica (numero di geni per megabase di sequenza), il numero di geni codificanti RNA non tradotti e il numero dei marcatori polimorfici per singolo nucleotide (SNP).
I dati sono aggiornati al maggio 2006 e tratti dalla banca dati EnsEMBL (si veda il Box 1.2).

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

POLIMORFISMO

RFLP

GAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATAT
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GACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCT**GCATTC**CCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACAC
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Allele 1

GAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATAT
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Allele 2

SNP

ACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTC
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Allele 1

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GAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGC

Allele 2

POLIMORFISMO

VNTR - STR

ACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTC
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CTC

Allele 1

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Allele 2

ACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTC
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GACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCT**GATAGATAGATAGATA**CCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGA
TATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCC
TGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGAC
CTGACACGTGCTAGCTAGCTCCTCTCGAGACGTAGGGCTCTCGATATAGCTCGCGACACACACAGATATATAGCGCTCCCTGAAACAGCTCCGACACAGCTCGCACACCGCTCGAGACCTGACCTGACACGTGCTAGCTAGCTCCTCTC

Allele 3

POLIMORFISMO CNV

5% del genoma umano

1

2

CNV = Copy Number Variation

- Del, ins, dupl:
  >1kb - parecchie Mb

- ~ 10.000
  Db of Genomic Variants

- ~ 5% genoma umano

- ~ 2900 geni

Nature 2006;444:444-454

Step fondamentali nello studio della variabilità genetica umana

APPLICAZIONI DEI POLIMORFISMI DEL DNA

MARCATORI GENETICI

Analisi di linkage

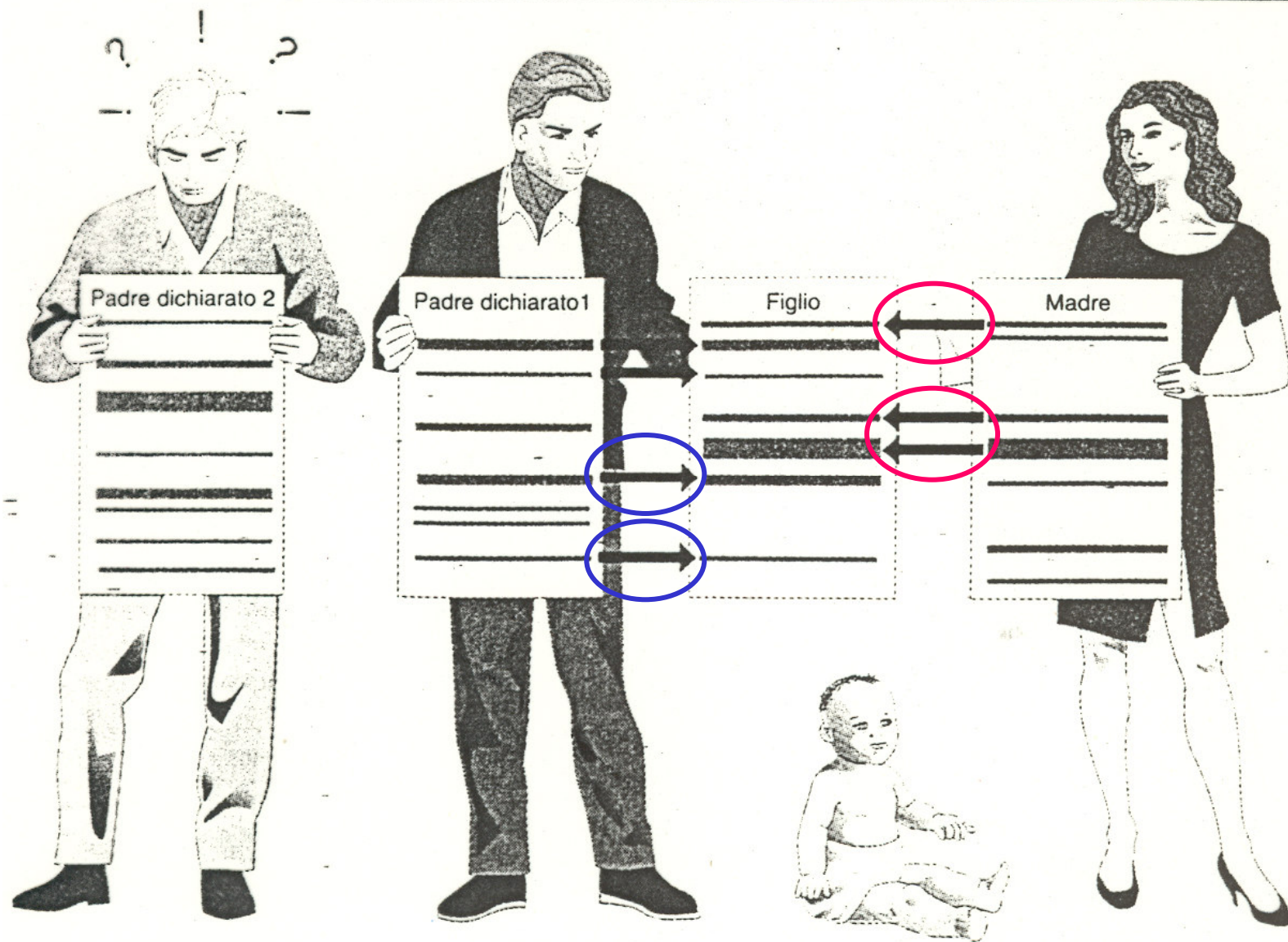
- Identificare geni – malattia (DMD, HD, CF – diagnosi portatore)
- Mappaggio sia genetico sia fisico

  genetico → ordinam. geni chr

  fisico → det. distanza fisica tra geni

IDENTIFICATORI INDIVIDUALITA"

- Controllo relazioni parentali in fam. con m. mendeliane
- Genetica di popolazione
- Indagini di paternità — Sangue periferico, ossa, saliva, capelli
- Indagini criminalistiche — Tracce biologiche (sangue, capelli, sperma, saliva, ossa)
- Controllo chimerismo — Sangue periferico, midollare
- Mola idatiforme — Sangue periferico, tessuto mola

Indagine di paternità

Indagini di paternità

Gruppi sanguigni
- ✓ABO, Rh, Duffy, Kidd, Lutheran, etc.

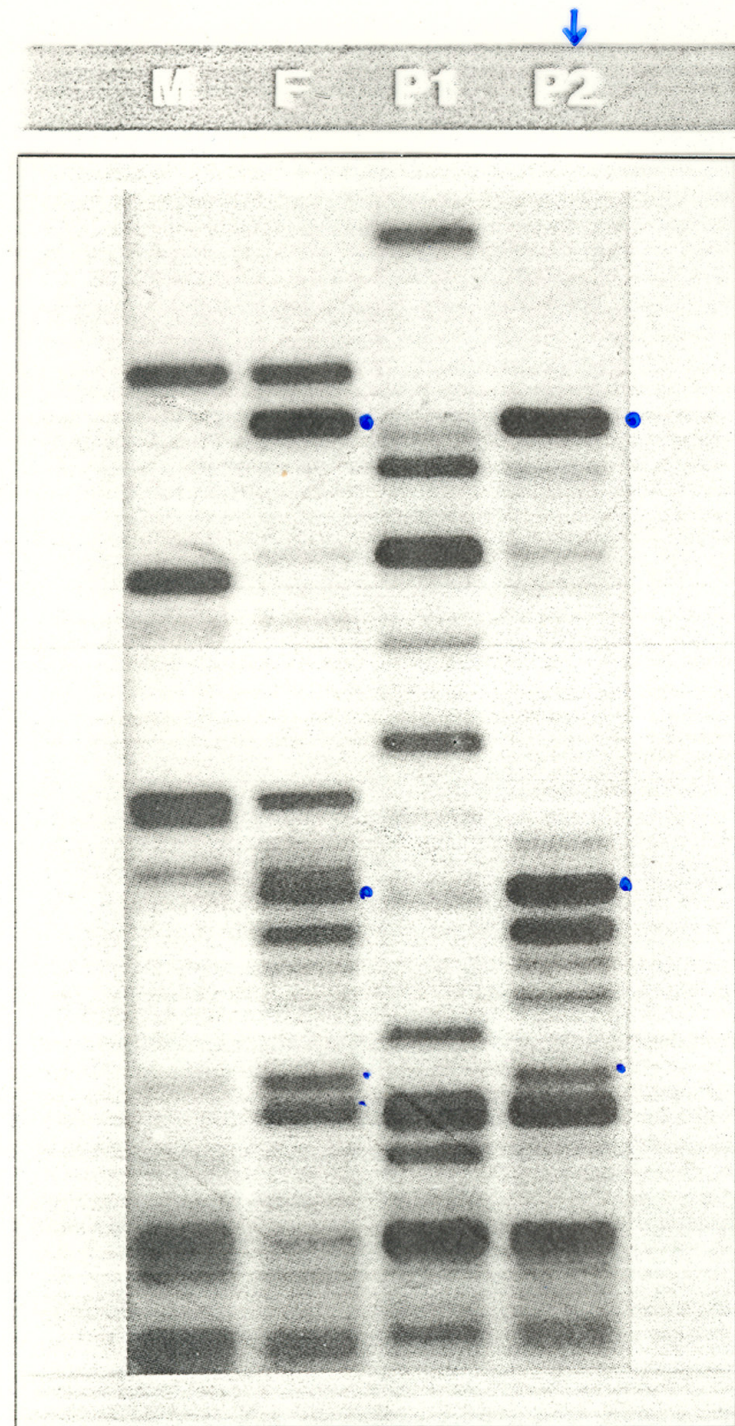
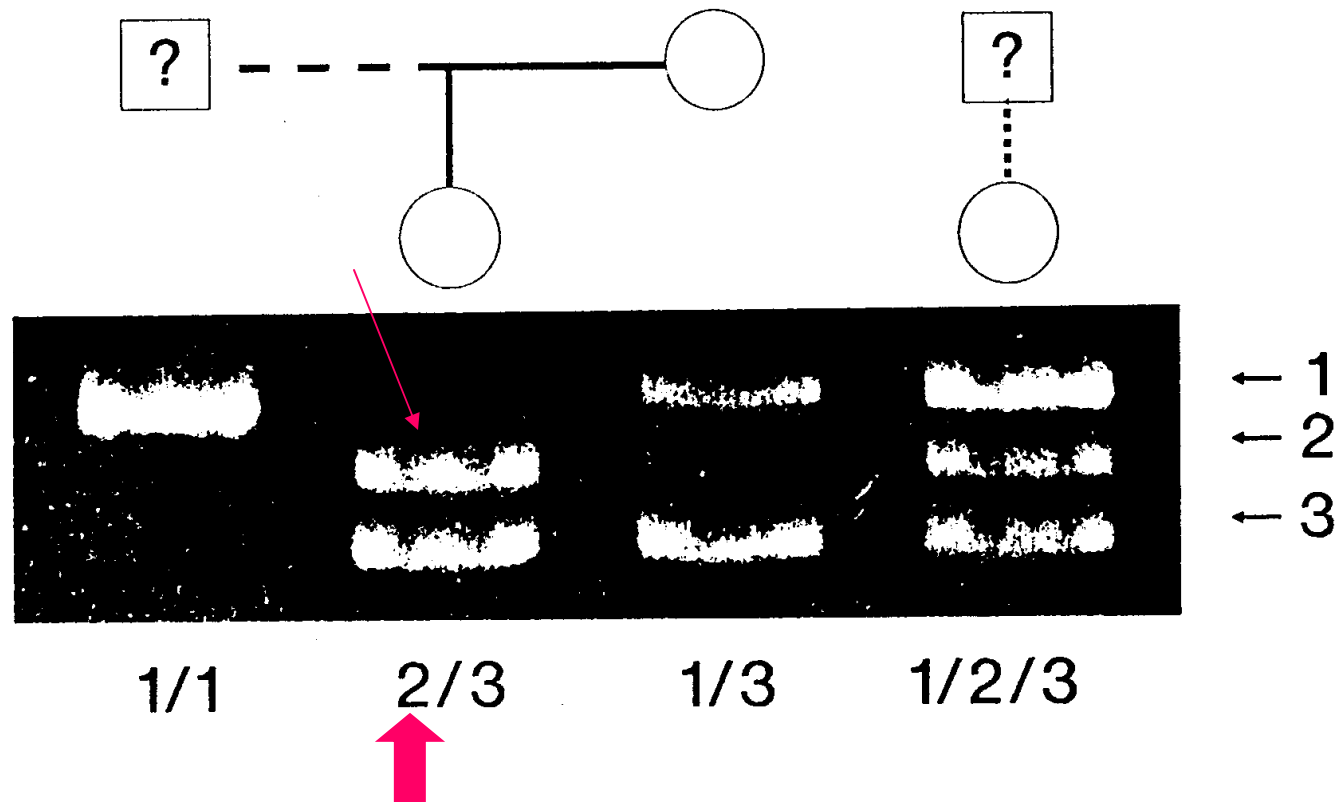
Polimorfismi sierici
- ✓Hp, Gc, Tf, Pi, Bf, etc.

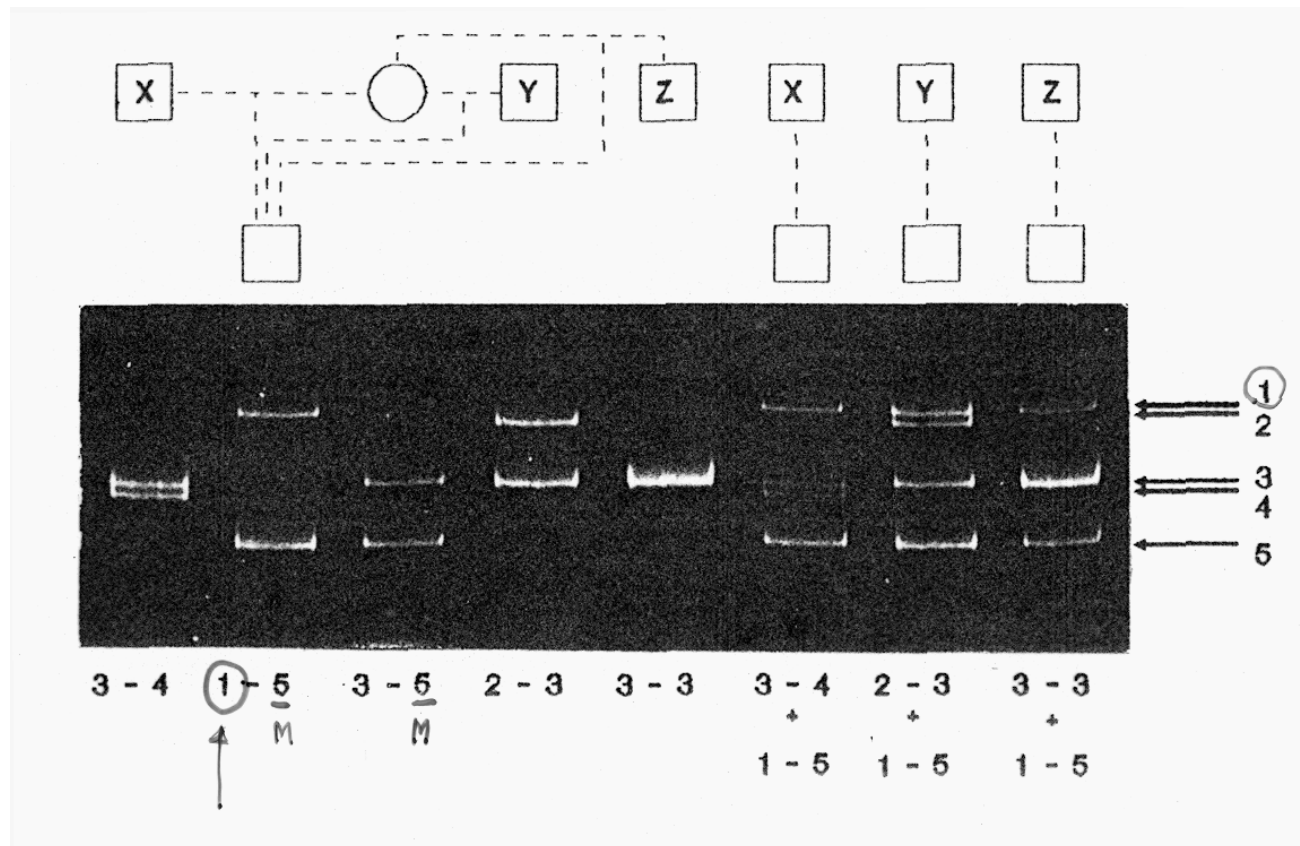
Polimorfismi enzimatici eritrocitari
- ✓GPT, PGM, EsD, AK, etc.

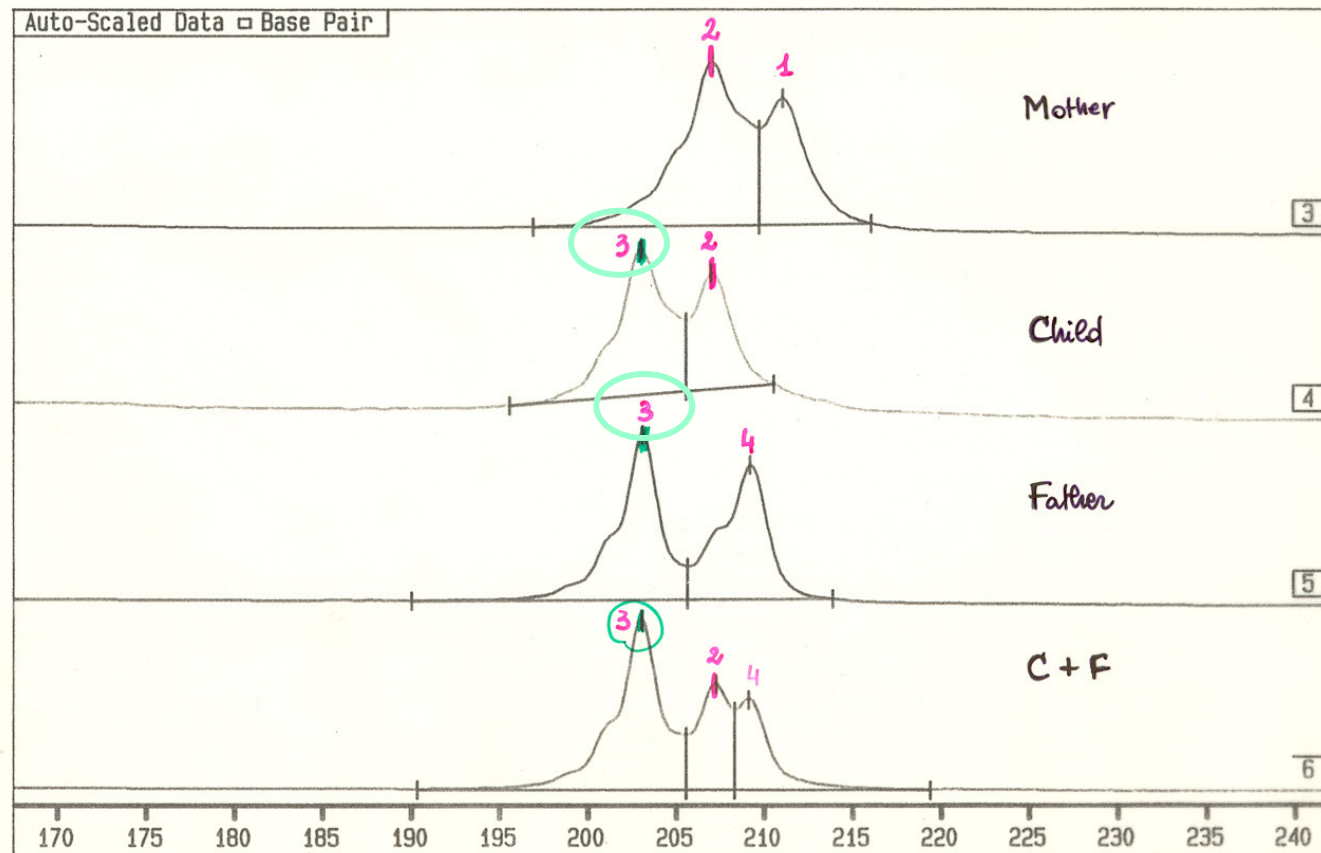
VNTR minisatelliti
- ✓YNH24, D17S5 (YNZ22),
- ✓D1S80 (MCT118), etc.

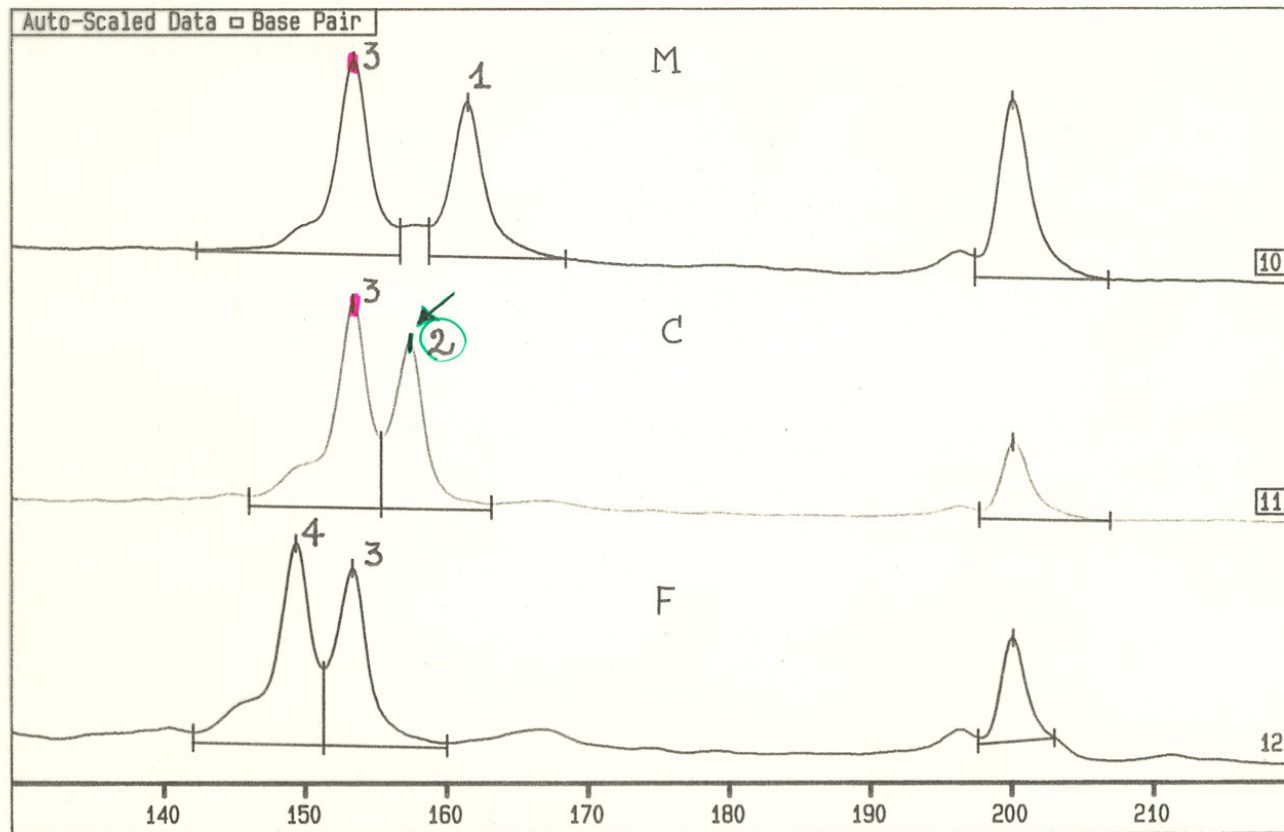
VNTR microsatelliti
- ✓D4S424, DYS19, D5S500, etc.

Attribuzione di paternità

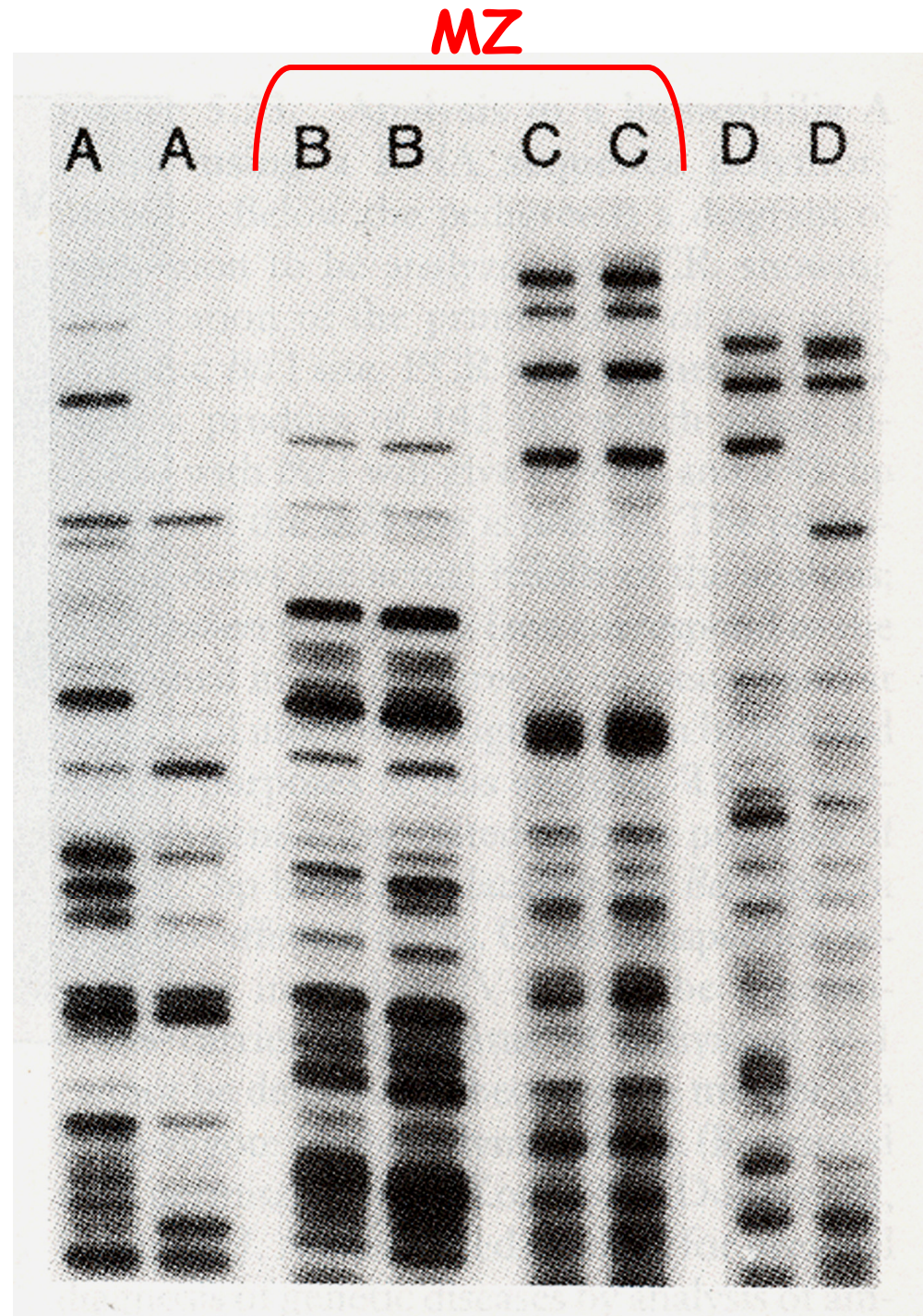
- 2 pP: P1 e P2
- > n° di bande condivise tra P e F

Indagine di paternità
VNTR D1S80

Esclusione di paternità

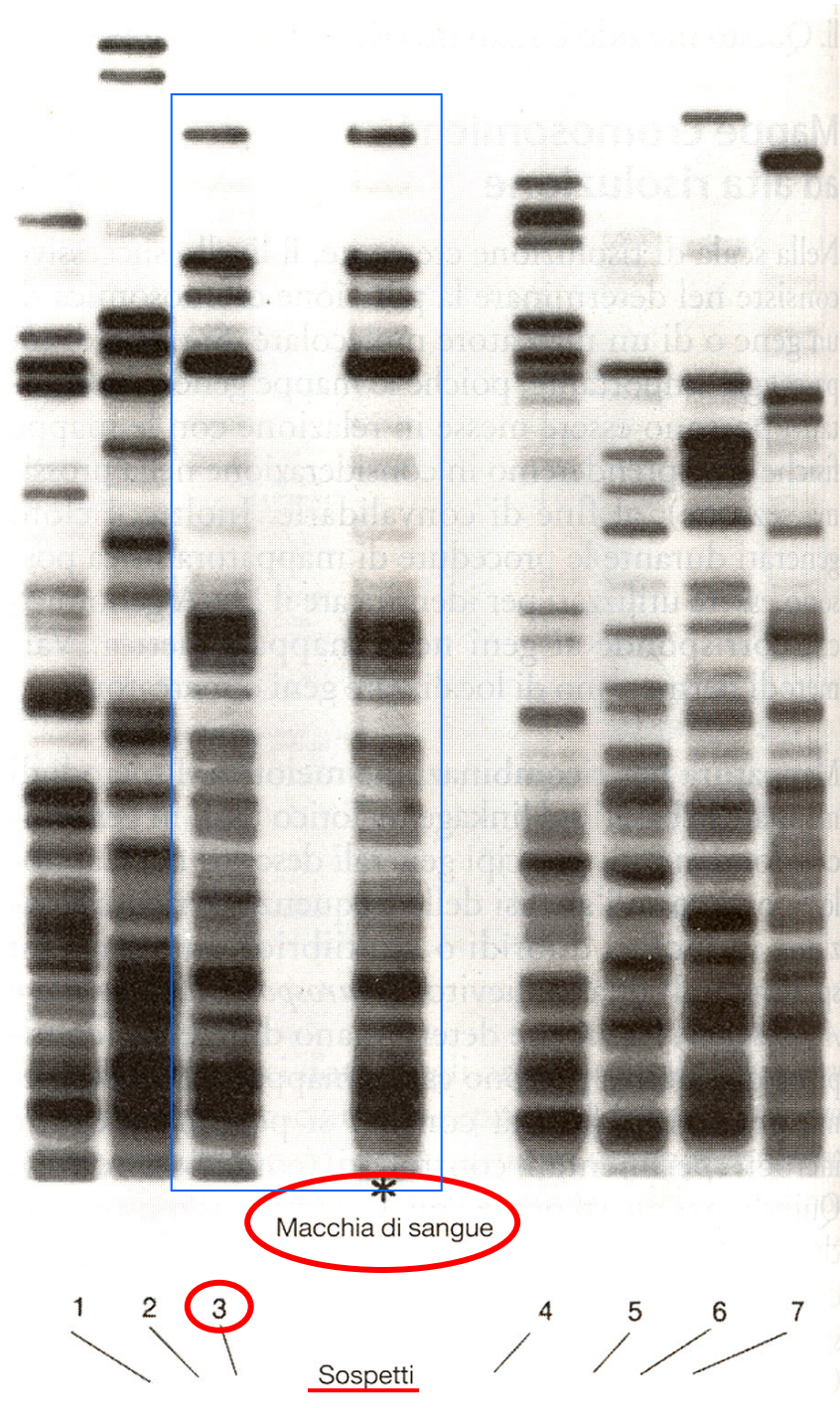
Indagine di paternità
VNTR APOB

Esclusione di paternità

Indagine di paternità
STR D5S500

Attribuzione di paternità

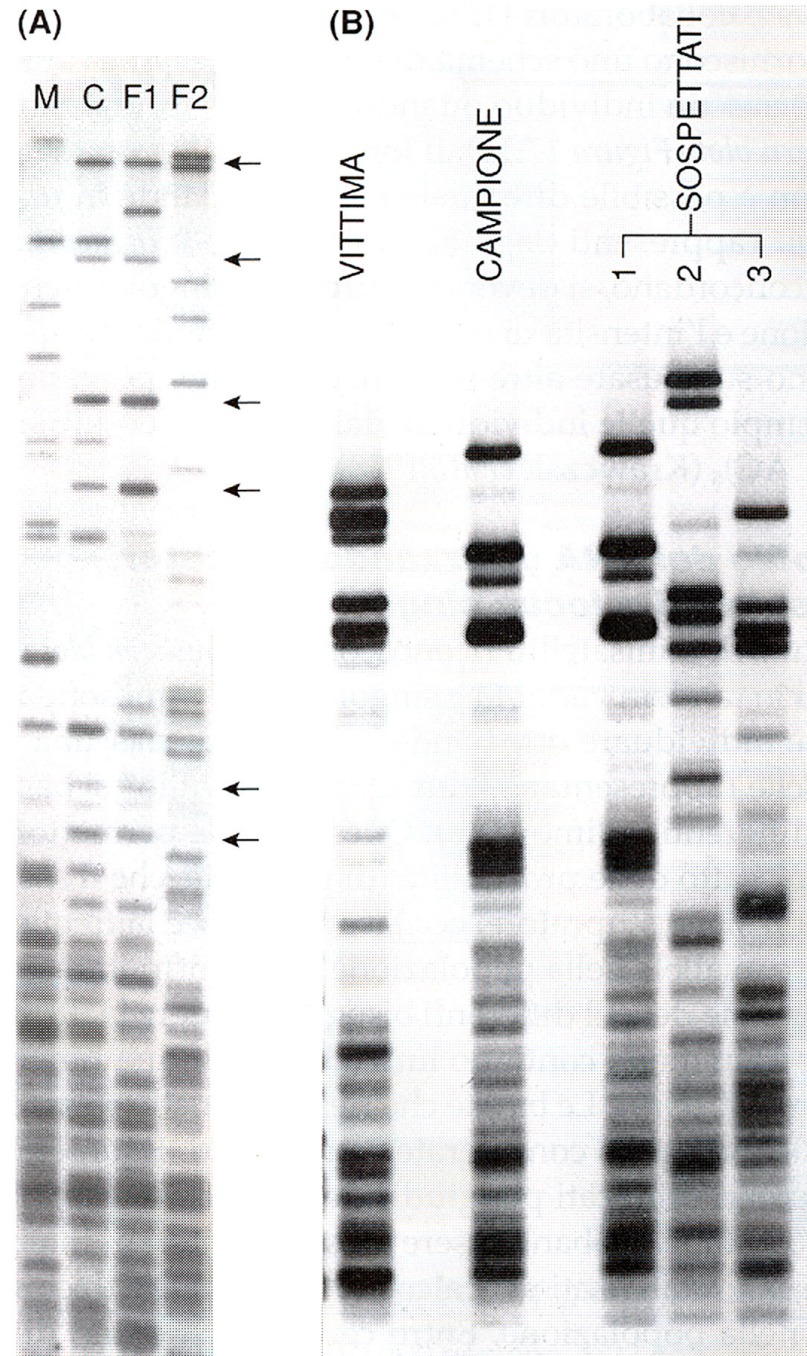
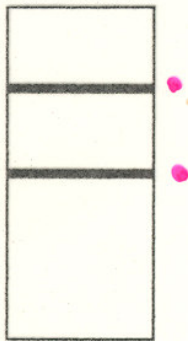
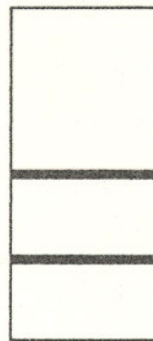
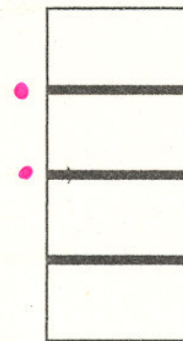
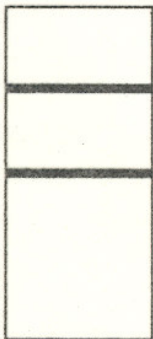
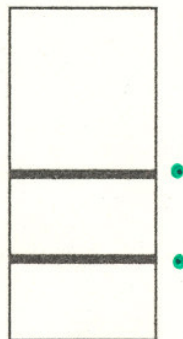
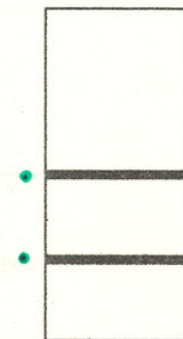
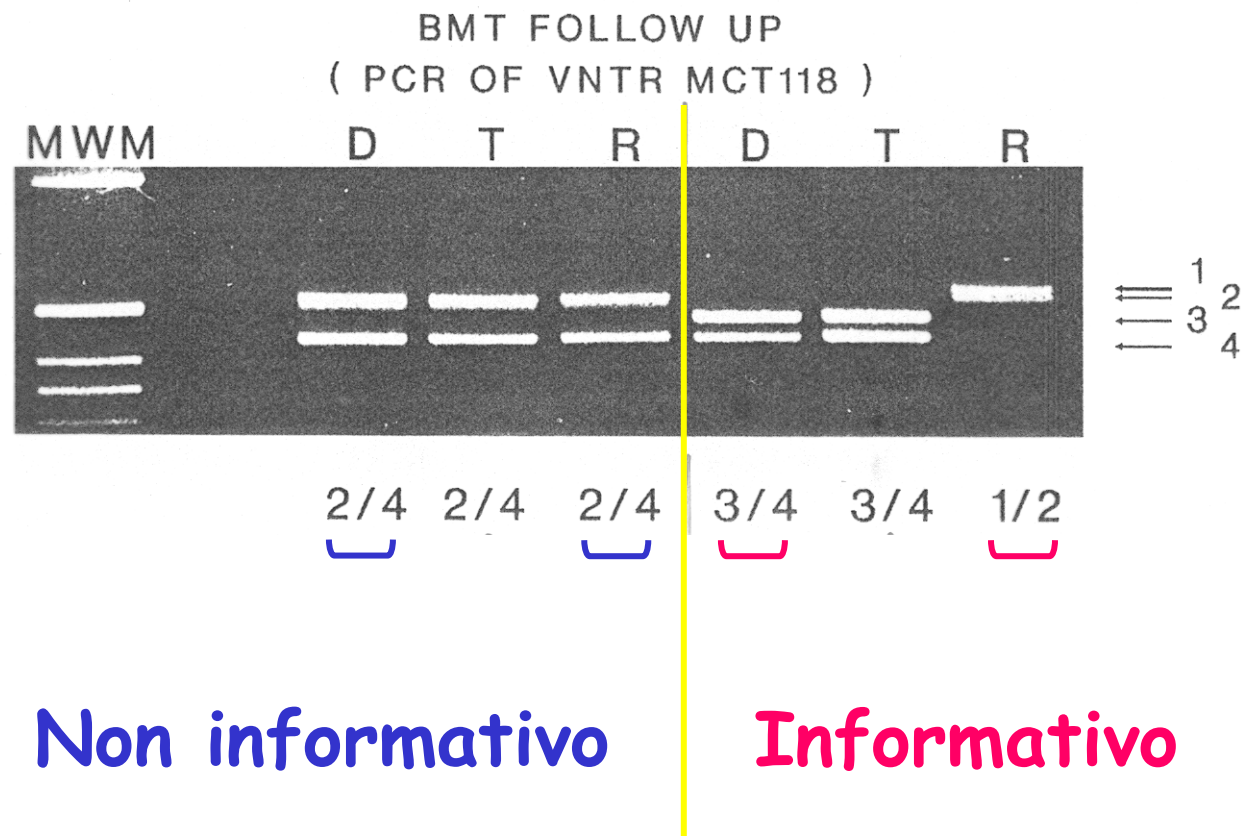
Indagine di paternità
VNTR HUMFES/FPS

Esclusione di paternità

DNA Fingerprinting su coppie di gemelli

Una sonda di DNA che identifica numerosi polimorfismi VNTR dispersi in numerose regioni del genoma

Comparazione dei *profili* di DNA in una indagine giudiziaria

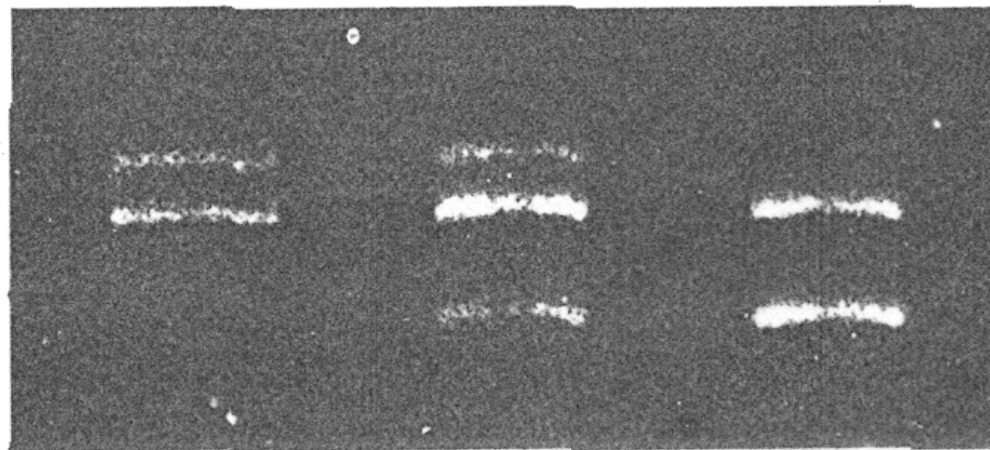
Sonda multi-locus DNA fingerprinting

DNA Fingerprinting

(A) Indagine paternità

(B) Indagine criminalistica

CHIMERISMO NEI TRAPIANTI DI MIDOLLO OSSEO
Donatore
Ricevente
Trapiantato
chimerismo
misto
Ricevente
Donatore
Trapiantato
chimerismo
completo

Informatività di un polimorfismo VNTR per la determinazione del chimerismo dopo BMT

MIXED CHIMERISM AFTER BMT

(PCR OF VNTR MCT118)

D T R

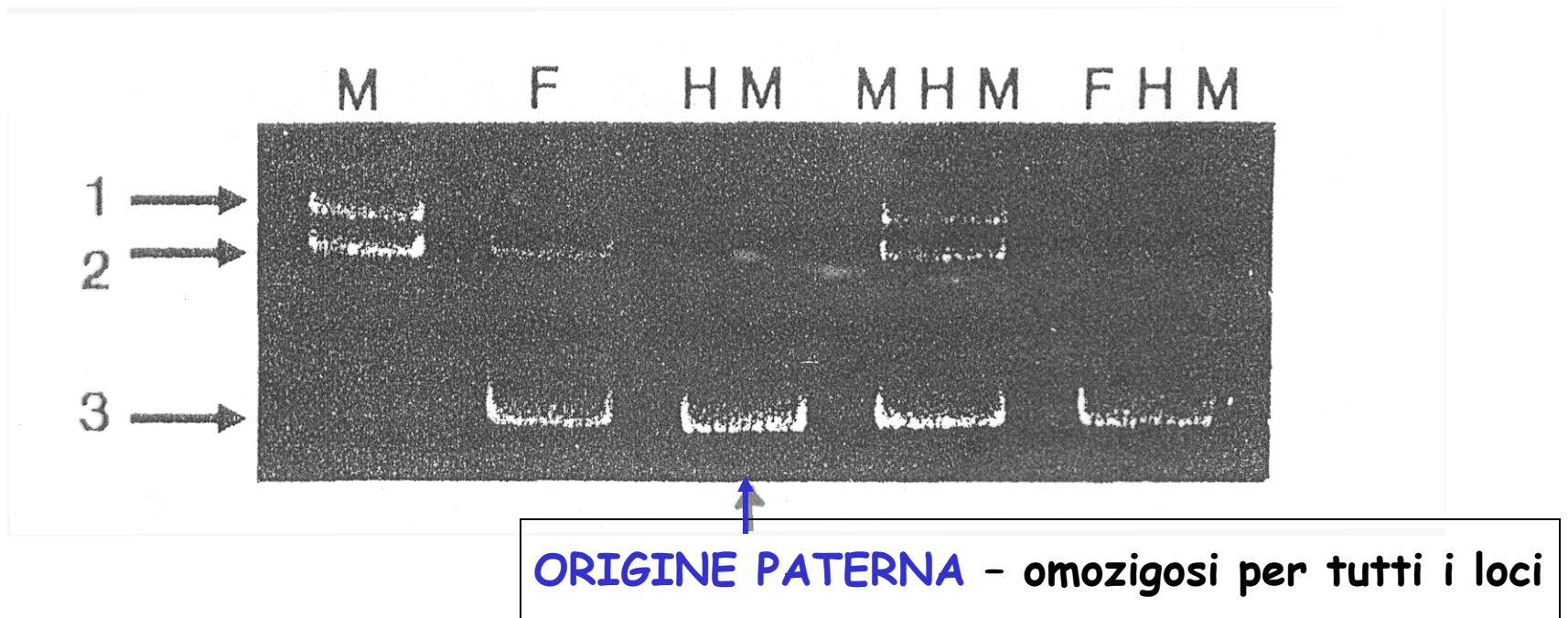
1
2
3

1/2 1/2/3 2/3

MOLA IDATIFORME (HM)

Disomia uniparentale (i chr. derivano da 1 unico genitore)
Cariotipo apparentemente normale (46, XX)

M F HM MHM FHM

1
2
3

ORIGINE PATERNA – **omozigosi per tutti i loci**

Ipotesi: degenerazione pronucleo femm. dell'uovo fecondato

Duplicazione del DNA del pronucleo maschile